

In the Specification

Please replace the title on page 1, line 1 with the following title:
INHIBITION OF APOPTOSIS IN KERATINOCYTES BY A LIGAND OF p75 NERVE
GROWTH FACTOR RECEPTOR.

Please replace the paragraph at page 39, lines 1 through 11 with the following paragraph.

Purified phosphorothioate oligonucleotides were purchased from Quality Controlled Biochemicals, Inc. (Hopkinton, MA). [19 mer] 19-mer oligonucleotides were designed based on the published human BCL-2 sequence (Tsujimoto, Y. and Croce, C.M., Proc. Natl. Acad. Sci. USA, 83:5214-5218 (1986)). The sequence chosen was directed against the 5' end of the coding region starting 4 bases before the methionine initiation site. Nonsense oligonucleotides were used as control. Sequences used (all written 5'-3'): Antisense CCCAGCGTGCGCCATCCTT (SEQ ID NO: 7); Nonsense CTCCCACTCGTCATTCGAC (SEQ ID NO: 8).

Please replace the paragraph at page 39, line 12 through page 40, line 10 with the following paragraph.

MM4 cells were maintained in 60 mm diameter tissue culture dishes in 55.3% DME, 27.6% L15, 15% FBS, 1% nonessential amino acids (GIBCO BRL), 2 mM glutamine and 10 µg/ml insulin. Near confluent cells were UV irradiated with 10 mJ/cm². Immediately after irradiation cells were incubated with 10 uM antisense or nonsense BCL-2 oligonucleotides in suspension at 37°C for 30 minutes. Then cells were plated in tissue culture dishes in the presence or absence of NGF (50 ng/ml). Cells were supplemented with fresh oligonucleotides every 12 hours. Cell yield and BCL-2 level were determined 48 hours after irradiation. Cell yield was determined by counting cells in at least three representative field per each condition. Figure 8 shows the results of a Western blot demonstrating that in the presence of antisense BCL-2 oligonucleotides BCL-2 levels are almost undetectable. Cell yields of UV irradiated cultures supplemented with NGF and nonsense oligonucleotides (white bar) are significantly higher as compared to nonsense supplemented cultures provided with diluent alone (dotted bar)

($p < 0.007$, ANOVA). Cell yields of NGF supplemented cultures treated with BCL-2 antisense oligonucleotides (black bar) are significantly lower than NGF supplemented cultures provided with nonsense oligonucleotides (white bar) demonstrating complete abrogation of NGF effect on the cells ($p < 0.003$, ANOVA). In diluent supplemented culture yields of nonsense treated cells (dotted bar) were significantly higher than antisense treated cells (dashed bar) ($p < 0.004$, ANOVA). Morphologic appearance of MM4 cells confirmed the numerical cell yield data. This experiment demonstrated that BCL-2 protein is required for melanocytic survival after UV irradiation and that NGF affects melanocytic cell survival by upregulating their BCL-2 level.

Please replace the paragraph at page 40, line 13 through page 41, line 6 with the following paragraph.

Cells were washed with cold PBS and disrupted in lysis buffer pH 8 (10 mM [tris] Tris, 150 mM [NaCl] NaCl, 0.1 mM EDTA, 1% SDS, 200 $\mu\text{g/ml}$ proteinase K). After 15 hour incubation at 37°C, samples were extracted twice with phenol plus chloroform (1:1, V/V) and precipitated overnight with ethanol (2.5 X volume) and 3 M sodium acetate (1/10 x volume). The DNA was then digested with DNase free ribonuclease (10 $\mu\text{g/ml}$) for one hour at 37°C, separated on 1% agarose gel and stained with ethidium bromide. The data show that DNA fragmentation, characteristic of apoptotic cell death, occurs in UV-irradiated but not sham irradiated keratinocytes.

Please replace the paragraph at page 41, lines 9 through 30 with the following paragraph.

Keratinocytes were UV-irradiated as in Example 9. After irradiation cells were placed in fresh keratinocyte medium containing 50 ng/ml NGF or diluent alone. DNA fragmentation was determined as in Example 9. Figure 9A shows that UV irradiated keratinocytes supplemented with diluent alone (-) display the characteristic DNA fragmentation, while DNA of UV irradiated cells supplemented with NGF (+) is not fragmented. The standard (STD) is 100 bp DNA ladder (Gibco/BRL). Keratinocyte yield determined daily for 5 days as shown in Figure 9B demonstrates that within 24 hours there is a 50% decrease in cell yield in cultures provided with

diluent alone but [on] only 30% decreases in cultures provided with NGF. UV irradiated keratinocytes were growth arrested as expected. However, cell yields of keratinocytes maintained in NGF supplemented medium increased by 40% within the 5 days of the experiment, suggesting that NGF is a mitogen for keratinocytes as well as a survival factor. This experiment demonstrates that, similar to melanocytes, NGF is a survival factor for keratinocytes. Furthermore, the experiment suggests that NGF might be a mitogen for keratinocytes as well.

Please replace the paragraph at page 46, lines 27 through 29 with the following paragraph.

p75^{NTR}-NIH 3T3 cells were [maintain] maintained in DMEM supplemented with 10% FBS in the presence of penicillin (45 ng/ml), streptomycin (68 ng/ml), and hygromycin B (17.5 ng/ml).

Amendments to the specification are indicated in the attached "Marked Up Version of Amendments" (pages i - iii).

In the Claims

Please amend Claim 6. Amendments to the claims are indicated in the attached "Marked Up Version of Amendments" (page iii).

6. (Amended) A method of preventing hair loss in a mammal comprising inhibiting apoptosis in epidermal keratinocytes.

REMARKS

Claim 6 has been amended.

Objection to the Specification

The Examiner has objected to the disclosure, stating that the title of the invention is not descriptive. The title has been amended as the Examiner suggested.